

REMARKS

Claims 27-32, 34-38, 43, 44, 46, 48 and 51-53 were pending. Entry of this amendment does not involve any issue of new matter, and are fully supported by the specification as described below. Applicant respectfully requests entry of this amendment.

Rejection under 35 USC 112, first paragraph

The Office Action rejects claims 27-38, 43, 44, 46, 48, and 51-53 under 35 U.S.C. §112, first paragraph as allegedly failing to comply with the enablement requirement.

The Examiner concedes that the specification enables methods of enhancing neuronal survival in the hippocampus in vivo by administering OP-1 to a subject. The Examiner alleges that the claims directed to method of reducing memory dysfunction associated with damaged hippocampal tissue are not enabled because memory function requires reconnecting the damaged neurons and reestablishing synaptic plasticity and cognitive function of the brain.

First, Applicant points out that the claims are directed method for reducing memory dysfunction associated with damaged hippocampal tissue caused by permanent or transient global ischemia. As such, "reconnecting the damaged neurons" may not necessarily involve physical axonal growth. Inventiveness of the claimed invention lies, in part, on the finding that morphogens can enhance cognitive function, even in the absence of overt physical injury to neurons. In ischemic conditions and neurotoxin exposure, many cells are damaged rather than killed and remain in the periphery of the lesion of cell death. For example, in Example 9, the specification states that "the MCA model is a reasonably predictive of the ability and efficacy of drugs, such as morphogen, to alter functional recovery in humans in whom central nervous system tissue has been damaged or lost due to stroke." (paragraph [131]). In addition, as shown in the "Bioassay" section (paragraphs [159]-[179]), upon administration of morphogens, cells exhibit changes in biochemical characteristics (N-CAM expression) and dendrite growth. Dendrite density correlates with retention of memory function in normal aging brain. (Morrison et al., Science 278: 412-419 (1997))

Further, even as to the Examiner's contention, it has been previously shown by Applicant's colleagues that morphogens indeed enhance synaptic regeneration in vitro (See US Pat. No.

6506729, especially Example 16) and, in fact, nerve gap repair was seen. (ibid., Example 9).

Therefore, Applicant submits that OP-1's activity to enhance neuronal connection has already been demonstrated. The present application relates to an analogous observation for damages caused by ischemia. Applicant submits that the claims are fully enabled considering the disclosure in the specification and the knowledge of one skilled in the art.

With regard to the types of morphogens including a mature form of OP-1 that are useful for the practice of the claimed methods, claims 34 and 35 were amended to recite the amino acid residues that comprise the mature OP-1 peptides. As previously explained, this recitation is supported by US Pat. No. 5266683 (the "'683 patent"), which is incorporated by reference. The Examiner contends that, because multiple forms of OP-1 are disclosed in the '683 patent, the support is not adequate. However, upon careful reading of the '683 patent, only the form having amino acid residues 293-431 of human OP-1 is referred to as "mature OP-1"; other forms are referred to as a "short form of OP-1" and "N-terminally truncated mature OP-1". They may all be morphogenetically active, but are clearly differentiated from the mature form of OP-1. The mature OP-1 protein is also described as having 139 amino acid residues. (Column 7, lines 9-10). Thus, Applicant submits that there is a clear and unambiguous support in the '683 patent for the recitation of "mature OP-1." For clarity, the claims have been amended to recite the actual amino acid residue numbers.

The independent claims recite morphogenic proteins with certain homology and identity to the active fragment of OP-1. Applicant respectfully directs the Examiner's attention to claims of patents already issued to Applicant and colleagues, for example, U.S. Pat. No. 6,565,843; 6,531,445; 6,194,376; 6,288,031; 6,077,823; 6,800,603; 6,407,060; 6,949,505; 6,498,142; 6,861,404; 6,090,776; 7,060,680; 5,674,522; 7,056,882; and 6,723,698. The claims of these patents all recite a morphogen analog with 70% homology or 60% identity to the seven-cysteine region of OP-1, and peptides having generic and other sequences related to OP-1. While Applicant is aware each application is examined independently, Applicant submits that these issued claims are presumed valid. As such, the burden is on the Examiner to establish that these claims are indeed not

enabled. Applicants also would like to remind the Examiner that predictability and consistency is beneficial and necessary among related patents.

Claim rejection under 35 USC 102(e)

The Office Action rejects claims 27-38, 43, 44, 46, 48, and 51-53 under 35 U.S.C. 102(e) alleging that they are anticipated by U.S. Patent No. 6,723,698 (the "698 Patent").

The Office Action alleges that examples 16.2, 17 and 18 of the '698 Patent anticipate the claimed invention. Under MPEP 2131.01, a reference must expressly or inherently describe every element of a claim to anticipate it.

The claims have been amended to recite a step of determining whether a mammal exhibits memory dysfunction. The support for this amendment can be found in Section IV Behavioral Assays, describing a test for declarative memory in humans and other additional tests for non-human mammals such as rats. Such step is not disclosed or suggested by the '698 patent. Including such a step in the method is clearly supported by the disclosure in the specification and knowledge of one in the art, as no need for such treatment exists if there is no memory dysfunction. Nowhere in the '698 patent is found disclosures about the effectiveness of a morphogen to reduce memory dysfunction. In fact, the '698 patent makes no reference to cognitive function. Therefore, the '698 patent fails to teach all the features of the pending claims and therefore it fails to anticipate them.

In view of the arguments set forth above, applicants respectfully request reconsideration and withdrawal of this ground of rejection.

Claim rejection under 35 USC 103(a)

The Office Action rejects claims 27-38, 43, 44, 46, 48, and 51-53 under 35 U.S.C. 103(a) alleging that they are made obvious by Rueger et al. (the '698 patent cited above) in view of Contestabile et al. (J. Neurosci. Res. 1990, 26: 483-7), Simonsen et al. (Scand. J. Work Environ. Health. 1994, 20: 1-12) and Gillette-Guyonnet et al. (Am. J. Clin. Nutr. 2000, 71: 637S-642S).

As described above, claims have been amended to recite the step of determining the existence of memory dysfunction. The '698 patent does not teach or suggest the association of memory dysfunction with hippocampal neuronal damage. Contestabile et al. describes formation of

lesion using ibotenic acid, but does not teach or suggest such a lesion with memory dysfunction nor specify the effect of hippocampal lesion. Ammonia is not disclosed. Simonsen et al. is a general thesis about neurotoxins including formaldehyde, but again does not associate any particular hippocampal damage with memory dysfunction. Gillette-Guyonnet describes weight loss including that by anorexia in Alzheimer's Disease patients, but states the reasons for such loss is unknown. The first cause hypothesized by the authors is atrophy of mesial temporal cortex, not hippocampus. Anorexia and Alzheimer's Disease may coexist, but there is no clear causal relationship determined yet.

Applicant has amended claim 48 to clarify that the hippocampal damage is caused by metabolic diseases, anorexia, or malnutrition. Paragraph [0009] supports this amendment, reciting malnutrition as a source of brain tissue damages, and that such malnutrition can be caused by metabolic diseases or anorexia, among others.

Applicant submits that, the references, alone or in combination, do not make obvious claims as amended. Accordingly, Applicant respectfully requests this ground of rejection be withdrawn.

Rejection under 35 USC 112, second paragraph

Claims 34 and 35 were rejected under 35 USC 112, second paragraph for allegedly being indefinite. Claims 34 and 35 were amended as described above in the section addressing the enablement rejection. Applicant submits that, as amended, in view of the remarks regarding the '683 patent, the claims are unambiguous and definite.

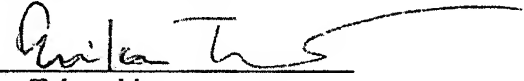
In view of the arguments set forth above, applicant respectfully requests reconsideration and withdrawal of this and all grounds of rejection addressed above.

CONCLUSION

No fee is deemed necessary in connection with the filing of this amendment. Authorization is hereby given to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 18-1945, under Order No. JJJ-P02-510 from which the undersigned is authorized to draw.

Dated: September 26, 2007

Respectfully submitted,

By 
Erika Takeuchi
Registration No.: 55,661
ROPES & GRAY LLP
12 11 Avenue of the Americas
New York, NY 10036
(212) 596-9000
(212) 596-9050 (Fax)
Attorneys/Agents For Applicant

Life and Death of Neurons in the Aging Brain

John H. Morrison* and Patrick R. Hof

Neurodegenerative disorders are characterized by extensive neuron death that leads to functional decline, but the neurobiological correlates of functional decline in normal aging are less well defined. For decades, it has been a commonly held notion that widespread neuron death in the neocortex and hippocampus is an inevitable concomitant of brain aging, but recent quantitative studies suggest that neuron death is restricted in normal aging and unlikely to account for age-related impairment of neocortical and hippocampal functions. In this article, the qualitative and quantitative differences between aging and Alzheimer's disease with respect to neuron loss are discussed, and age-related changes in functional and biochemical attributes of hippocampal circuits that might mediate functional decline in the absence of neuron death are explored. When these data are viewed comprehensively, it appears that the primary neurobiological substrates for functional impairment in aging differ in important ways from those in neurodegenerative disorders such as Alzheimer's disease.

The neuropathologic changes associated with neurodegenerative disorders invariably reflect the following events: (i) major circuits are structurally disrupted through synapse loss and neuron death, (ii) there is selective vulnerability in respect to which neurons die and which are resistant to degeneration, and (iii) the symptoms of the particular neurodegenerative disorder follow from the circuits that are disrupted and thus reflect the selective vulnerability. In the case of Alzheimer's disease (AD), neuronal degeneration is not only reflected by neuron and synapse loss in certain areas but also by neuropathologic profiles such as the neurofibrillary tangles (NFT) and senile plaques (SP), which are considered a prerequisite for the neuropathologic diagnosis of the disease and reflective of degeneration (1). Although NFT and SP are reflective of cellular pathology and as such are abnormal, their mere presence may not be sufficient to account for the dementia of AD (2). Both NFT and SP are also present in cognitively normal aged individuals, but in AD, the distribution and density of NFT and SP must be such that they reflect a major disruption of key cortical circuits (3).

The neocortex and hippocampus are both devastated in AD, but the pathology is not ubiquitous, nor does it affect all cell

types. The pyramidal cells in the entorhinal cortex and the CA1 and subiculum regions of the hippocampus are vulnerable to NFT formation and resultant degeneration, whereas the CA3 region and the granule cells in the dentate gyrus are resistant to degeneration (3). In the neocortex, particular subsets of pyramidal cells are prone to NFT formation and degeneration, whereas others are not, and inhibitory interneurons do not form NFT and are largely resistant to degeneration (3, 4). The pyramidal cells that furnish long corticocortical projections are thought to be particularly vulnerable to degeneration in AD, leading to a global disruption of interconnections between association cortices, whereas primary sensory and motor areas exhibit minimal neuron loss (3, 5). There is extensive synapse loss in association areas as well, further reflecting structural disruption of circuits (6). With respect to subcortical projections, most of the specific thalamic projections remain intact, whereas the cholinergic projection from the nucleus basalis of Meynert degenerates early in the disease (7). Thus, although degeneration is extensive in AD, it is selective, and given the broad array of circuits that degenerate, it is not surprising that multiple domains of cognition are disrupted, including memory and attention.

Human Entorhinal Cortex: The Interface Between Aging and AD

In respect to AD and aging, the single most vulnerable circuit in the cerebral cortex is the projection referred to as the perforant path, which originates in layer II of the entorhinal cortex (EC) and terminates in the outer molecular layer of the dentate

gyrus, thus providing the key interconnection between the neocortex and the hippocampus (8). The EC is a region of extraordinary convergence of inputs from the association cortex, essentially funneling highly processed neocortical information into the dentate gyrus of the hippocampus and thereby playing a crucial role in memory (9, 10) (Fig. 1). This circuit is invariably devastated by extensive NFT formation in AD, even at the earliest stages of the disease (11). The layer II neurons of the EC are rich in neurofilament protein in the healthy state, but even after normal aging, a few of these neurons are invariably in transition to NFT when viewed in double-labeling immunohistochemical experiments localizing the cytoskeletal proteins neurofilament and tau (Fig. 2). In fact, it appears that the vast majority of humans older than 55 years have at least a few NFT or neurons in transition to NFT in layer II of the EC (3, 12–15). These individuals are likely to be asymptomatic with no obvious memory loss. Even the most rigorous analysis of neuron loss would not reveal the transitional neurons as "missing," because they represent only a few percent of the total number of neurons and likely still appear normal in the Nissl stains used to estimate total neuron number.

Despite these similarities, there are qualitative and quantitative differences between normal aging and AD with respect to NFT formation and neuron loss in EC. These contrasts are revealed most clearly in studies involving stereological techniques to estimate neuron number in key hippocampal and neocortical regions. These procedures have been recently reviewed in some detail (16). Perhaps the most important advantage is that these techniques allow one to obtain an accurate estimate of the number of neurons within a given brain structure without relying on a density measurement that would be confounded by changes in the size of neurons or the size of the structure under investigation, either through fixation parameters or the aging process itself (Fig. 3). Using a stereological design, Hyman and colleagues recently estimated the degree of neuron loss in clinically characterized subjects (15, 17). In neurologically normal elderly individuals, there was no evidence of neuron loss in any layer of the EC (15), even though as stated above (13), there were likely a few neurons in transition to NFT in layer II of the EC in these brains (Figs. 2 and 4). However, in subjects characterized as having very mild AD [for example, with a clinical dementia rating score of 0.5 (18)], there was already a quite extensive loss of neurons in the EC, as much as 50% of the neurons from layer II of EC, suggesting that the perforant path would be

J. H. Morrison is with the Neurobiology of Aging Laboratories, the Fishberg Research Center for Neurobiology, and the Department of Geriatrics and Adult Development, Mount Sinai School of Medicine, New York, NY 10029, USA. P. R. Hof is with the Neurobiology of Aging Laboratories, the Fishberg Research Center for Neurobiology, the Department of Geriatrics and Adult Development, and the Department of Ophthalmology, Mount Sinai School of Medicine, New York, NY 10029, USA.

*To whom correspondence should be addressed. E-mail: morrison@cortex.neuro.mssm.edu

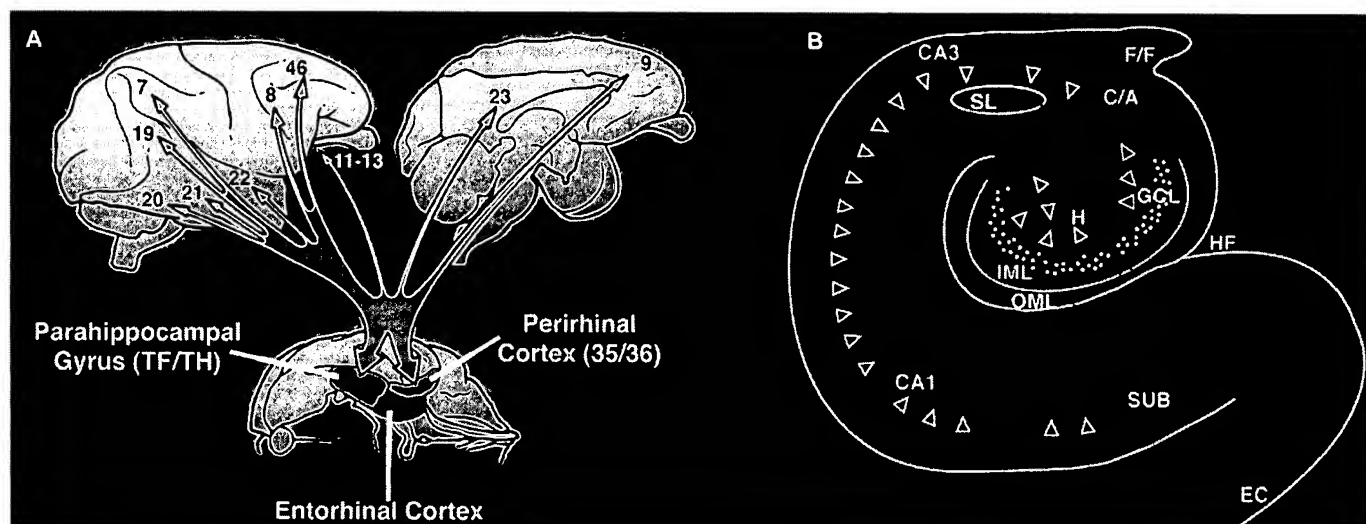


Fig. 1. (A) Connectivity of the parahippocampal gyrus (areas TF and TH), perirhinal cortex (areas 35 and 36) and EC with other cortical regions. This diagram demonstrate the massive convergence of cortical inputs onto cortical areas of the medial temporal lobe (thick yellow arrows), which receive a widespread array of topographically organized projections from most regions of the neocortex and send more discrete reciprocal projections (thin yellow arrows) to association areas throughout the neocortex [adapted from Squire and Zola-Morgan (9)]. Neocortical regions on the hemisphere are numbered following Brodmann's nomenclature. **(B)** Schematic representation of corticocortical connectivity within the hippocampal formation demonstrating elements of the trisynaptic circuit (in red). These elements include the perforant pathway (PP) projection from layer II of the EC to the outer molecular layer of the dentate gyrus (OML), the mossy fiber projection (MF) from the dentate granule cells to the stratum lucidum (SL) of the CA3 field, and the Schaffer collaterals (SC) innervating the pyramidal neurons in the CA1 field. Also shown are commissural and associational afferents (C/A) from the fimbria/fornix (F/F) and the output projection from the subiculum (SUB) to the deep layers of the EC. GCL, granule cell layer of the dentate gyrus; H, hilus; HF, hippocampal fissure; IML, inner molecular layer of the dentate gyrus. [Panel (B) courtesy of S. J. Siegel and A. H. Gazzaley]

partially disrupted in these cases. In severe AD, almost 90% of the neurons in layer II of EC are missing, with many presumably now having degenerated as end-stage NFT. In addition, both the neurologically normal and mild AD cases had no detectable neuron loss in the temporal neocortex, which agrees with other studies demonstrating that normal or mildly impaired aged individuals do not have significant NFT formation in neocortical areas (3, 14, 19). This observation is in striking contrast to AD where NFT are present throughout association cortices with particularly high densities in prefrontal and temporal cortices.

Thus, there are quantitative differences between normal aging, mild AD, and severe AD in that particularly vulnerable areas such as the EC have increasing numbers of neurons lost, and there are qualitative differences in that regions such as the prefrontal cortex do not exhibit significant neuron loss in normal aging but are likely to in AD (Fig. 4). In fact, the dementia of AD that goes well beyond memory loss in its functional impact is unlikely to emerge before incipient involvement of neocortex (in particular, the inferior temporal neocortex), and the severity of dementia correlates more with neocortical NFT counts than with EC or hippocampal NFT counts (3, 14). Finally, with respect to the NFT formation that occurs in normal aging, we should not assume that this minor degree of

NFT formation is a predecessor to the more severe degeneration that is present even in early AD. There is no definitive evidence supporting such a progression (20, 21). In addition, when placed in the context of data on normal aging, it is unlikely that the functional decline in memory as a result of normal aging is a result of this minimal neurofibrillary pathology occurring in the EC.

Neurofilament Protein: A Molecular Link to Neurodegeneration

It is likely that the vulnerable neurons in neurodegenerative disorders share key biochemical and cellular properties that are linked to their vulnerability; one such property may be their particular cytoskeletal profile. In numerous neurodegenerative disorders, affected neurons often display characteristic neurofibrillary inclusion bodies that are considered hallmarks of a particular disease process. For example, Lewy bodies in neurons within substantia nigra are diagnostic of Parkinson's disease, Lewy bodies in cerebral cortex are associated with certain forms of dementia, Pick bodies in cerebral cortex are the defining neuropathologic lesion in Pick's disease, and amyotrophic lateral sclerosis (ALS) exhibits neurofilament-protein-containing spheroids and inclusions in motor neurons (3, 22). In AD,

NFT occur primarily in neurofilament-protein-rich neurons, and in these susceptible neurons, cytoskeletal elements dynamically aggregate into NFT (Fig. 2) (13, 23). Also, the microtubule-associated protein tau is a major constituent of the pathologic accumulation of fibers referred to as paired helical filaments in NFT (22, 24) (Fig. 2).

Therefore, whereas different groups of neurons degenerate in these diseases, they are similar in the extent to which cytoskeletal alterations are a defining attribute of the degenerative process. Transgenic mouse models that overexpress the heavy or light subunits of neurofilament protein display motor neuron disease similar to ALS, as do transgenic mice that contain one of several mutations of superoxide dismutase (SOD) that have been demonstrated to occur in familial ALS (25). Interestingly, even in the SOD model of ALS, it is the neurons that have a particularly high expression of neurofilament protein that are most vulnerable (26). In addition, transgenic mice that express the mid-sized subunit of human neurofilament protein exhibit multiple pathologic profiles reminiscent of spheroids, NFT, and Pick bodies, further supporting a key role for neurofilament protein in neurodegeneration (27).

These data, taken together, suggest that neurofilament protein synthesis, degradation, assembly, or transport in neurons where such processes are highly regulated

may be implicated in neurodegeneration across a broad spectrum of diseases and linked to individual patterns of selective vulnerability. Such a role for neurofilament protein does not negate an equally important role in neurodegeneration for oxidative stress and aberrant calcium metabolism, and in fact, these processes may even augment disruption in neurofilament protein assembly or transport (28).

Normal Aging and Neuronal Viability

Until recently, it was widely accepted that neuron death was an inevitable result of normal aging. This conviction was spawned primarily by a few influential papers from as early as the 1950s that demonstrated significant neuron death in aged humans in the absence of AD, as well as in nonhuman primates and rodents (29, 30). These studies reported disparate results with respect to degree of neuron loss, but as a group, their data suggested that most neocortical areas and certain hippocampal subfields lose 25 to 50% of their resident neurons with age.

These studies virtually all shared one design characteristic: the investigators were measuring neuron density in a given structure, not total neuron number. When the field was rigorously reviewed by Coleman and Flood in 1987 (30), the inescapable conclusion from the literature was that there was extensive neuron loss with aging, although it was already clear to these authors that the data might be confounded in several cases by species and strain differences, tissue processing, and sampling design.

With the development of more accurate procedures for counting neurons (16) (Fig. 3), this view has been modified over the last several years, particularly as stereological procedures for estimating neuron number have been applied to aging research (15–17, 21, 31–34). The careful application of stereological techniques to several species, including humans, have led to the somewhat surprising conclusion that age-related decline in neuron number through neuron death is not significantly involved in normal aging, at least with respect to the neocortex and hippocampus.

A recent stereological analysis of total

neocortical neuron number in humans revealed sex- and age-related differences in neuron number, but sex was found to be a much more powerful predictor of total neocortical neuron number than age, and although the authors found a 10% decrease in neurons across the age spectrum, they warn that, given the study design, this small but significant decline across ages should not be interpreted as strong evidence for biologically significant age-related neuron decline (33). Studies of total neocortical neuron counts are difficult to put into a clear functional context, given the enormous regional, laminar, and cellular heterogeneity that exists in neocortex. Thus, the more revealing studies with respect to aging have been those that have analyzed a given brain region that can be equated with certain functions and connections. For example, in aged rats there does not appear to be any significant neuron loss in any of the major regions of the hippocampus or EC that could account for the age-related memory defects that occur. Data from nonhuman primates show no evidence of age-related neuron loss in the various hippocampal subfields, particularly in layer II of the EC and in CA1; therefore, neuron loss cannot account for any disruption, structural or functional, of the perforant path input to the dentate gyrus from the EC, or for disruption of hippocampal output from CA1. In addition, there is no neuron loss in nondemented elderly humans in the EC or CA1, the two hippocampal regions most directly implicated in memory function, but there is some age-related neuron loss in the hilus of the dentate gyrus and the subiculum (15–17, 34).

Even though there does not appear to be neuron loss that would structurally compromise the key hippocampal circuits mediating memory, there are decrements in functions that are reliant on these circuits. Numerous behavioral tasks that are demonstrated to be specific for hippocampal function have revealed a memory defect associated with age in both rodents and nonhuman primates (35, 36). There are also strong data supporting similar memory loss in aged humans (37), and recent data suggest that age-related memory impairment in humans is quite different from that seen even in early AD (38). The major difference with early AD patients is that after a delay, healthy elders retain new information, whereas patients with mild AD retain little new information (38). This impairment in information retention, which characterizes the early stages of AD, is correlated not only to neuronal loss in the EC but also to changes in volumetric indices within the temporal lobe, as revealed by neuroimaging studies (38). The impairment

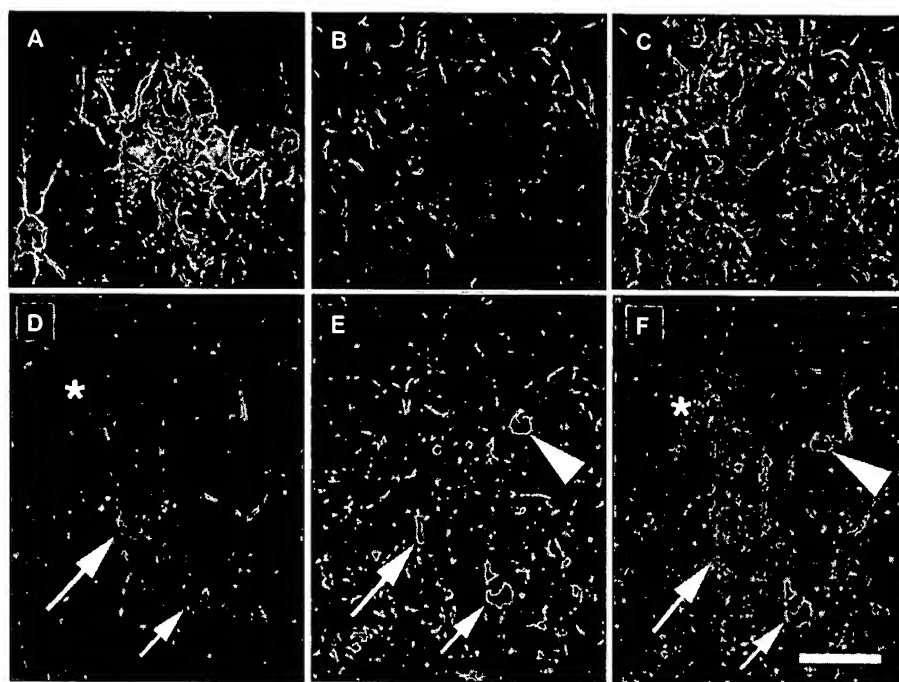


Fig. 2. Progression of changes in neurofilament-protein-enriched neurons in AD. In the early stages of AD, intensely neurofilament-protein-immunoreactive neurons in layer II of the EC (A) begin to form NFT, as demonstrated by the presence of hyperphosphorylated tau protein (B). At this stage, most of the layer II neurons are affected by NFT formation but still retain neurofilament-protein immunoreactivity (double labeling) (C). As AD progresses, these neurons eventually lose neurofilament-protein immunoreactivity (13). In a more advanced AD case, transitional forms can be observed in layer III of the superior frontal cortex (D through F). Several neurons contain both neurofilament-protein immunoreactivity (D) and tau immunoreactivity (E) [arrows; yellow fluorescence on (F)], whereas a few neurons have lost their neurofilament protein immunoreactivity and contain only a tau-positive NFT [arrowhead (E and F)]. Also, healthy neurons that do not yet contain a tau immunoreactivity are still visible [asterisk (D and F)]. Tissues were stained with a monoclonal antibody to neurofilament protein and a polyclonal antibody to the microtubule-associated protein tau as in (13). Scale bar = 80 μ m.



is not specifically related to chronological age—in all three species, a significant proportion of aged subjects did not display age-related memory impairment—which is particularly relevant to the concept of distinguishing “normal” from “successful” aging (39). The study by Rapp and Gallagher (32) that provided stereologic data from the hippocampus of behaviorally characterized aged rats showed directly that neuron death is not likely to be the cause of functional decline. In a subset of these animals where a memory deficit had been demonstrated, there was no decrease in the number of neurons in the various hippocampal subfields when compared to unimpaired aged rats or to young rats. Parallel results have been reported in nonhuman primate models of normal cognitive aging (31, 32).

Anatomically, there have been age-related changes reported that are short of neuron death. Geinisman has reported loss of synapses in the perforant path terminal zone of the dentate gyrus that could affect function of this circuit, but this does not appear to occur in monkeys (40). In addition, in both rodents and humans, changes have been reported in dendritic arbor, spines, and synapse morphology that could impact on the function of hippocampal circuits but would not be reflected as neuron loss (30, 41). With respect to prefrontal

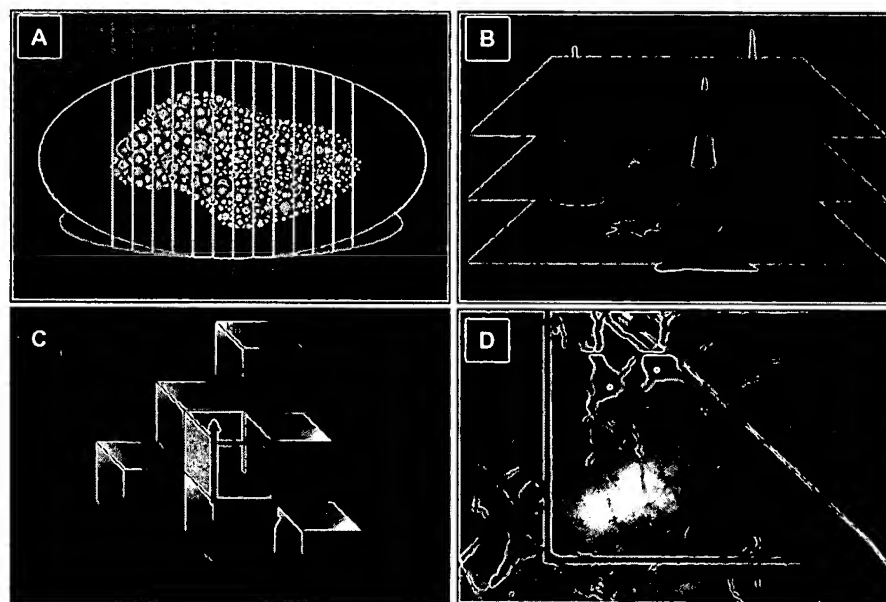
cortex, Peters and colleagues have described aged monkeys with no neocortical neuron loss but demonstrable cognitive impairment, which they hypothesize to be linked to disruption of the myelin sheath in the subcortical white matter of these monkeys (42), suggesting that the connections to and from the prefrontal cortex might be intact but functionally compromised.

Electrophysiological investigations of aging in the rodent hippocampus have revealed that many aspects of synaptic transmission are unaffected or even compensatory, whereas others are compromised (43), supporting the anatomic studies in suggesting that aging does not involve a general deterioration or frank degeneration of circuits. One component of synaptic transmission that does appear to be compromised is maintenance of long-term potentiation (LTP), particularly in the dentate gyrus, and induction of LTP under certain circumstances, both of which could be related to memory defects (43, 44). Perhaps the most exciting result regarding electrophysiological assessment of age-related changes in hippocampal function is the recent report by Barnes and colleagues of a decreased stability of spatial information coded by “place cells” in aged rats as compared to that in young rats (36) (Fig. 5A). Place cells are neurons within the CA1 region that

maintain a spatial map of the rat's position within a familiar environment. Interestingly, place cells in aged animals function normally while in the familiar environment; the failure is in their ability to retrieve the same map upon revisiting the environment, essentially offering a striking cellular substrate for spatial disorientation that would be manifested as “getting lost.” The authors hypothesize that this deficit in recalling spatial representations is linked to the age-related disruption of LTP that has been demonstrated in rodents (36).

Given that structural degeneration of hippocampal circuits is not necessary to account for such impairments in hippocampal function, one might hypothesize that more subtle molecular changes in intact circuits crucial for memory processes contribute to age-associated memory impairment. The important roles of both the perforant path and *N*-methyl-D-aspartate (NMDA) receptors in memory processing and LTP have been extensively documented (45). A recent report on aged monkeys demonstrated a circuit-specific decrease in expression of NMDA receptors that could possibly serve as a substrate for memory defects or deficits in LTP (46). The NMDA receptor levels were decreased specifically in the outer molecular layer of the dentate gyrus, where the perforant path terminates

Fig. 3. Some principles of unbiased quantitative neuropathology. Neuropathologic specimens are seldom exhaustive, because they usually consist of several sections through the structure in question at one or two levels and are therefore not representative of the entire object. However, when the entire object is sampled systematically, the analysis becomes representative of the whole structure. (A) A three-dimensional array of particles of varying sizes and densities. An exhaustive series begins at or before the beginning of the object and ends when the object ends. From this series, every *n*th section may be used for counting, yielding a manageable number of slides. This technique requires access to the entire object and the ability to recognize its borders. (B) Another type of bias stems from the use of relatively thin histological sections and their treatment as essentially two-dimensional planes. As an example, consider an array of neurons. Each time a neuron is intercepted by a plane, it produces a two-dimensional profile. Counting these profiles, as is still commonly done, leads to the following problem. As sectioned in (B), all neurons are sampled; however, some are sectioned, and therefore counted, more than once. If the interval between sections were doubled (by omitting the middle plane), small neurons would go uncounted. The probability of a neuron's being counted is proportional to its height, and thus, even in widely spaced sections, this method leads to overcounting of larger neurons and undercounting of smaller ones. The way to avoid this source of bias is to count not on planes but in volumes. This method does not entail the generation of new sections, merely a new way of looking at the same old ones. (C) The recognition of the need to count in volumes led to the development of the disector (54). A disector is simply a volume of tissue in which objects are counted according to simple



rules. Counting in disectors thus consists of sampling three-dimensional boxes within each section and counting within them. (D) As applied to microscopy, this technique amounts to sampling across the surface of a histological section and counting in the depth of each site at high magnification. The counting frame depicted in (D) is an unbiased counting frame (54). The yellow line in (D) shows the outline of the structure of interest. For more details on the use of the unbiased counting frame and counting using the disector principle, see (54, 55). [Figure designed and provided by E. A. Nimchinsky]

(Fig. 5B), and there were no significant shifts in α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) or kainate glutamate receptor subunit proteins (46). In addition, both pre- and postsynaptic elements of the circuit itself were structurally intact, and in these same animals, it was demonstrated through stereological analysis that there was no decrease in the number of neurons in layer II of the EC (Fig. 5C) (31, 46). Thus, this shift in NMDA receptor expression in the perforant path terminal zone occurred in the absence of significant shifts in any other glutamate receptors and in the absence of any reflection of structural degeneration of this circuit.

Several transgenic mouse models that do not require any associated degenerative events have also offered indirect molecular links to potential age-related memory impairment (47). Although these animals have not been tested directly with respect to age-related memory decline, genetic manipulations of several NMDA receptor subunits and Ca^{2+} - and calmodulin-dependent kinase II have led to defects in LTP and learning in the absence of degenerative changes, similar to those hypothesized to occur in aging as a result of circuit-specific shifts in NMDA receptor proteins. These molecular links to functional decline are independent of the molecules implicated in mouse models of neurodegenerative disorders, such as neurofilament protein or SOD for ALS (25) and amyloid A β protein for AD (48).

Estrogen: A Neuroendocrine Link to Neural Aging

Although the neurodegenerative events underlying AD may be distinct from the events that mediate age-related memory impairment, estrogen may be a crucial mediator in both processes. McEwen and colleagues have demonstrated estrogen-induced increases in the density of spines on CA1 pyramidal cells that would likely impact on the strength of excitatory synaptic inputs to these neurons (49). In addition, these changes are NMDA receptor-dependent (49). More recently, it was demonstrated by Gazzaley and colleagues (50) that estrogen replacement therapy in ovariectomized rodents in fact increases the NMDAR1 protein concentration in CA1 pyramidal cell somata and dendrites as well as the somata of the dentate granule cells. Furthermore, electrophysiological data demonstrate an increase in NMDA-mediated responses in CA1 in precisely the same ovariectomy and estrogen replacement protocol in which the increase in NMDAR1 protein levels was observed in these neurons (51).

In addition, recent clinical studies of humans have demonstrated that estrogen has a protective effect in respect to the onset of AD (52). Although there are no data that speak directly to whether or not estrogen prohibits neuron death, the fact that it is protective against AD suggests that it protects against neurodegeneration. This role is further supported by *in vitro* experiments demonstrating that estrogen can protect neurons in culture against amyloid-induced toxicity or other excitotoxic events (53).

Thus, it would appear that estrogen might be a key player in both neurodegenerative events and nondegenerative events that lead to age-related impairment of memory and cognition. This effect is of obvious relevance to postmenopausal women, yet its relevance to similar age-related functional decline or the risk of AD in males is currently unclear. Links among

estrogen, NMDA receptors, hippocampal circuits, and memory represent a particularly fruitful area of investigation in gerontology and will likely emerge as a major focus in the neurobiology of aging over the next decade.

Conclusions

The emerging human and animal data suggest that although the dementia of AD involves the degeneration of key neurons and circuits, age-related memory impairment is likely to reflect more subtle structural alterations and molecular changes in specific neurons and circuits that mediate such functions, in the absence of significant degenerative events. This view also suggests that age-related memory impairment and AD are not part of a continuum, and the former does not necessarily reflect a predisposition for the latter. Both behavioral and

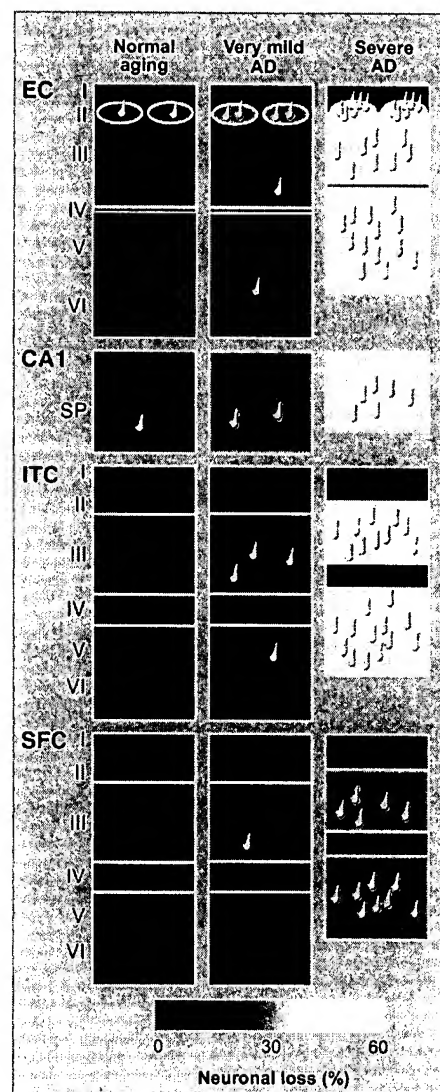


Fig. 4. Schematic representation of regional and laminar NFT formation and neuronal loss in normal aging and AD. The yellow flame-shaped structures represent a semiquantitative assessment of NFT densities. An estimate of the percent of neuronal loss is shown by the different shades of blue (scale at bottom). In normal aging, a few NFT are consistently observed in layer II of the EC and rare NFT are occasionally encountered in the stratum pyramidale of the CA1 field (SP). The inferior temporal cortex (ITC) and superior frontal cortex (SFC) remain devoid of NFT. There is no neuronal loss in normal aging. In contrast, very mild AD is characterized by higher NFT densities in the EC and CA1, and NFT are consistently observed in layer III of the ITC. Rare NFT are present in SFC. The neocortical areas show no neuronal loss, but a significant degree of neuronal loss is present in layer II of the EC and in the CA1 field. In severe AD, NFT are found in high densities in layer II of the EC, in the CA1 field, and in layers III, V, and VI of the ITC, with moderately high density in SFC as well. The degree of neuronal loss parallels NFT densities in these regions, although NFT numbers alone cannot account for the total loss of neurons, indicating that not all dying neurons necessarily undergo NFT formation. The size of the cortical boxes reflects a certain degree of tissue shrinkage in severe AD. Data used in this schematic were inferred from several sources (14–17, 19–21, 33, 34).



electrophysiological studies suggest that key hippocampal circuits are functionally compromised in a subset of aged rodents and nonhuman primates and that these functional declines do not reflect neuron loss. An important component of such molecular shifts in intact circuits may be the NMDA receptor, which has been demonstrated to exhibit circuit-specific decreases in dendritic levels that would likely impact the ability of the perforant path to mediate memory processing. Age-related shifts in LTP and inability to retain spatial maps

have also been demonstrated electrophysiologically in awake, behaving animals. Although the NMDA receptor-associated functional decline is clearly mechanistically independent of and less severe than memory loss due to circuit degeneration, estrogen may play a crucial role in both processes. Such multiple roles for estrogen would suggest that reproductive senescence may have a multifaceted impact on memory loss and cognitive decline through decreased regulation of intact hippocampal circuits as well as decreased protection of

such circuits from degeneration. It is perhaps not surprising that estrogen, a molecule that is so crucial to survival of the species through regulation of the female reproductive system, also plays a key role in the regulation of multiple neural processes that confer significant survival value. The relevance of such neuroendocrine influences on aging is less clear but probably profound for males as well. As research in the neurobiology of aging moves forward, we can expect progress in the determination of the mechanisms by

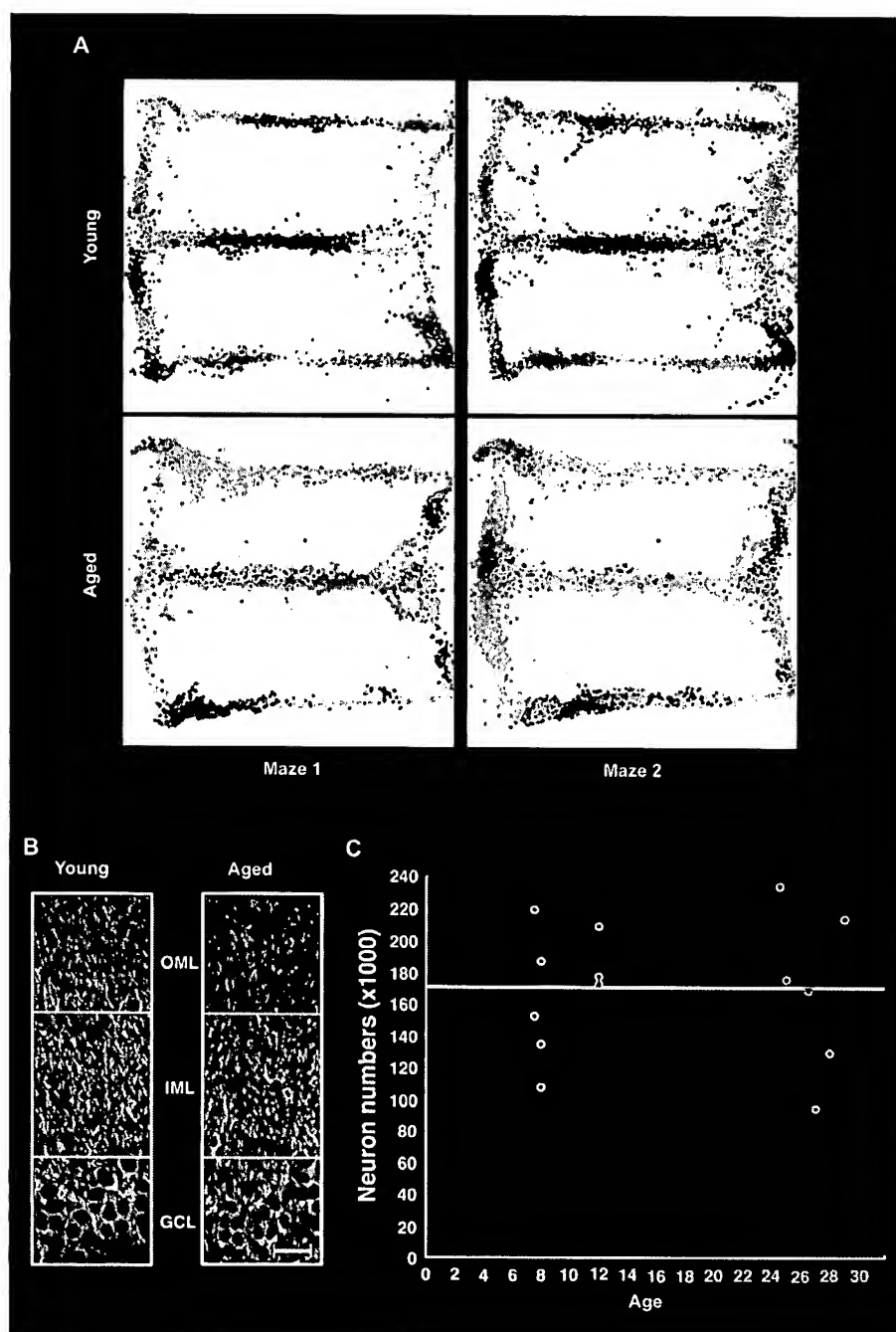


Fig. 5. Age-related changes in hippocampal NMDA receptors and electrophysiological properties. **(A)** Place-field distributions derived from simultaneous multiunit recordings from CA1 pyramidal cells from a young and an old rat recorded on two consecutive trials (Maze 1 and Maze 2) moving through a rectangular figure-eight maze. The gray lines indicate the trajectories of the rats, and each color corresponds to a single recorded cell, with each spike emitted from that cell represented by a dot in its designated color. Thus, in the resultant place-field map the portion of the maze dominated by a given color represents the portion of the maze in which that neuron is preferentially active. Note that in the young animal, the place-field maps are similar across the two maze episodes, as represented by the similar color schemes. In contrast, the color schemes are not highly correlated across episodes for the old animal, demonstrating an inability to retain the place fields from one episode to the next. [Adapted from Barnes *et al.* (36)] **(B)** Age-related changes in the perforant path terminal zone of a macaque monkey. In an aged macaque monkey, there is a consistent decrease in immunofluorescent staining intensity for the NMDAR1 subunit restricted to the outer molecular layer of the dentate gyrus (OML) compared to young animals, whereas no such changes are visible in the inner molecular (IML) and granule cell (GCL) layers. The images are pseudo-colored confocal images using the glow scale that recreates the heat scale as a way to illustrate relative staining intensities. Scale bar = 25 μ m. [Adapted from Gazzaley *et al.* (46)] **(C)** Stereologic estimates of total neuron numbers in layer II of the EC in the same animals that demonstrated the decrease in NMDAR1 immunofluorescence intensity in (B). Note that there is no loss of these neurons in aged monkeys compared to young ones, demonstrating that the NMDA receptor change has occurred in the absence of a structural degeneration of the perforant pathway, as exists in AD (31, 46).

which crucial neural circuits become vulnerable to degeneration and to nondegenerative routes to functional decline. Progress can also be expected in the identification of clinically useful molecular interventions, such as estrogen, that will augment the continued protection and proper function of these circuits.

REFERENCES AND NOTES

1. S. S. Mirra *et al.*, *Neurology* **41**, 479 (1991); S. S. Mirra, M. N. Hart, R. D. Terry, *Arch. Pathol. Lab. Med.* **117**, 132 (1993).
2. A panel of neuropathologists convened by the National Institute on Aging and the Ronald and Nancy Reagan Institute of the Alzheimer's Association recently developed a set of recommendations for post-mortem diagnosis of AD [*Neurobiol. Aging*, in press]. There are several key points made in this important document, among them that neurofibrillary pathology occurs in a limited fashion in many aged individuals that do not have dementia. Also, the degree to which these pathologic changes are diagnostic of AD is dependent on their regional location and density. Thus, given that NFT and neuropil threads can be present in neurologically normal individuals, and that their distribution and density constitute a more reliable correlate of AD than their absolute presence or absence, we prefer to consider such profiles as reflecting neurofibrillary pathology, rather than referring to them as AD-related changes or AD lesions.
3. P. R. Hof and J. H. Morrison, in *Alzheimer Disease*, R. D. Terry, R. Katzman, K. L. Bick, Eds. (Raven, New York, 1994), pp. 197-229; *J. Am. Geriatr. Soc.* **44**, 857 (1996); P. R. Hof, *Eur. Neurol.* **37**, 71 (1997); C. Bouras, J. H. Morrison, in *Neurodegenerative and Age-Related Changes in Cerebral Cortex*, vol. 13 of *Cerebral Cortex*, A. Peters and J. H. Morrison, Eds. (Plenum, New York, in press).
4. P. R. Hof and J. H. Morrison, *Exp. Neurol.* **111**, 293 (1991); P. R. Hof *et al.*, *J. Neuropathol. Exp. Neurol.* **50**, 451 (1991); P. R. Hof, E. A. Nimchinsky, M. R. Celio, C. Bouras, J. H. Morrison, *Neurosci. Lett.* **152**, 145 (1993); V. L. Sampson, J. H. Morrison, J. C. Vickers, *Exp. Neurol.* **145**, 295 (1997).
5. S. Mitrux, *Monatsschr. Psychiatr. Neurol.* **113**, 100 (1947); R. C. A. Pearson, M. M. Esiri, R. W. Hiorns, G. K. Wilcock, T. P. S. Powell, *Proc. Natl. Acad. Sci. U.S.A.* **82**, 4531 (1985); J. Rogers and J. H. Morrison, *J. Neurosci.* **5**, 2801 (1985); C. Duyckaerts *et al.*, *Acta Neuropathol.* **70**, 249 (1986); D. A. Lewis, M. J. Campbell, R. D. Terry, J. H. Morrison, *J. Neurosci.* **7**, 1799 (1987); P. R. Hof, K. Cox, J. H. Morrison, *J. Comp. Neurol.* **301**, 44 (1990); P. R. Hof and J. H. Morrison, *ibid.*, p. 55; M. C. De Lacoste and C. L. White III, *Neurobiol. Aging* **14**, 1 (1993); J. H. Morrison, *ibid.*, p. 51.
6. E. Masliah, R. D. Terry, M. Alford, R. DeTeresa, L. A. Hansen, *Am. J. Pathol.* **138**, 235 (1991); E. Masliah, M. Mallory, L. Hansen, R. DeTeresa, R. D. Terry, *Neurology* **43**, 192 (1993).
7. D. A. Drachman and J. Leavitt, *Arch. Neurol.* **30**, 113 (1974); P. Davies and A. J. F. Maloney, *Lancet* **ii**, 1403 (1976); J. T. Coyle, D. L. Price, M. R. DeLong, *Science* **219**, 1184 (1983); C. Geula and M. M. Mesulam, in *Alzheimer Disease*, R. D. Terry, R. Katzman, K. L. Bick, Eds. (Raven, New York, 1994), pp. 263-291.
8. D. L. Rosene and G. W. Van Hoesen, in *Further Aspects of Cortical Function, Including Hippocampus*, vol. 6 of *Cerebral Cortex*, E. G. Jones and A. Peters, Eds. (Plenum, New York, 1987), pp. 345-456; D. G. Amaral and M. P. Witter, *Neuroscience* **31**, 571 (1989); M. P. Witter, H. G. Groenewegen, F. H. Lopes Da Silva, A. H. M. Lohman, *Prog. Neurobiol.* **33**, 161 (1989); M. P. Witter, G. W. Van Hoesen, D. G. Amaral, *J. Neurosci.* **9**, 216 (1989).
9. L. R. Squire and S. Zola-Morgan, *Trends Neurosci.* **11**, 170 (1988).
10. ———, *Science* **253**, 1380 (1991).
11. B. T. Hyman, G. W. Van Hoesen, A. R. Damasio, C. L. Barnes, *Science* **225**, 1168 (1984); B. T. Hyman, G. W. Van Hoesen, L. J. Kromer, A. R. Damasio, *Ann. Neurol.* **20**, 472 (1986); B. T. Hyman, G. W. Van Hoesen, A. R. Damasio, *Neurology* **40**, 1721 (1990).
12. P. V. Ariagada, J. H. Growdon, E. T. Hedley-White, B. T. Hyman, *Neurology* **42**, 631 (1992); P. V. Ariagada, K. Marzloff, B. T. Hyman, *ibid.* **42**, 1681 (1992); J. H. Morrison *et al.*, *Brain Res.* **416**, 331 (1987).
13. J. C. Vickers, A. Delacourte, J. H. Morrison, *Brain Res.* **594**, 273 (1992).
14. P. R. Hof *et al.*, *Arch. Neurol.* **49**, 946 (1992); C. Bouras, P. R. Hof, J. H. Morrison, *Neurosci. Lett.* **153**, 131 (1993); C. Bouras, P. R. Hof, P. Giannakopoulos, J. P. Michel, J. H. Morrison, *Cereb. Cortex* **4**, 138 (1994); P. Giannakopoulos, P. R. Hof, S. Motter, J. P. Michel, C. Bouras, *Acta Neuropathol.* **87**, 456 (1994); L. M. Bieri *et al.*, *Arch. Neurol.* **52**, 81 (1995); P. Giannakopoulos, P. R. Hof, J. P. Michel, J. Guimon, C. Bouras, *Brain Res. Rev.*, in press.
15. T. Gómez-Isla *et al.*, *J. Neurosci.* **16**, 4491 (1996).
16. M. J. West and H. J. G. Gundersen, *J. Comp. Neurol.* **296**, 1 (1990); M. J. West, *Neurobiol. Aging* **14**, 287 (1993); M. J. West, P. D. Coleman, D. G. Flood, J. C. Troncoso, *Lancet* **344**, 769 (1994).
17. T. Gómez-Isla *et al.*, *Ann. Neurol.* **41**, 17 (1997).
18. C. P. Hughes, L. Berg, W. L. Danziger, L. A. Cohen, R. L. Martin, *Br. J. Psychiatry* **140**, 566 (1982).
19. H. Braak and E. Braak, *Acta Neuropathol.* **68**, 325 (1985); H. Crystal *et al.*, *Neurology* **38**, 1682 (1988); R. Katzman *et al.*, *Ann. Neurol.* **23**, 138 (1988); B. M. Hubbard, G. W. Fenton, J. M. Anderson, *Neuropathol. Appl. Neurobiol.* **16**, 111 (1990); H. Braak and E. Braak, *Acta Neuropathol.* **82**, 239 (1991); D. W. Dickson *et al.*, *Neurobiol. Aging* **13**, 179 (1992); J. C. Morris *et al.*, *Neurology* **41**, 469 (1991); J. L. Price, P. B. Davis, J. C. Morris, D. L. White, *Neurobiol. Aging* **12**, 295 (1991); L. Berg, D. W. McKeel, P. Miller, J. Baty, J. C. Morris, *Arch. Neurol.* **50**, 349 (1993).
20. In this context, epidemiologic, clinical, and neuropathologic studies have shown that the characteristics of brain aging in very old individuals differ considerably from those in patients younger than 90 years. The prevalence of dementia is much lower among very old individuals, and it has been suggested that the "oldest-old" population may in fact represent a genetically distinct group. In centenarians, the EC is relatively mildly affected in terms of neuron loss even in patients with AD, and the distribution of NFT and SP lesions in hippocampus and neocortex differs from that in younger individuals [J. J. Hauw *et al.*, *Rev. Neurol.* **142**, 107 (1986); T. Mizutani and H. Shimada, *J. Neurol. Sci.* **108**, 168 (1992); P. Delabarre, Y. He, G. Fayet, C. Duyckaerts, J. J. Hauw, *Neurobiol. Aging* **14**, 191 (1993); P. Giannakopoulos, P. R. Hof, M. Surini, J. P. Michel, C. Bouras, *Acta Neuropathol.* **85**, 602 (1993); P. Giannakopoulos *et al.*, *Dementia* **5**, 348 (1994); G. W. Rebeck *et al.*, *Neurology* **44**, 1513 (1994); P. Giannakopoulos *et al.*, *Arch. Neurol.* **52**, 1150 (1995); P. Giannakopoulos, P. R. Hof, C. Bouras, *Lancet* **346**, 1486 (1995); K. Ritchie and D. Klida, *ibid.*, p. 931; E. Sobel *et al.*, *Neurology* **45**, 903 (1995); T. Asada *et al.*, *J. Am. Geriatr. Soc.* **44**, 151 (1996); N. T. Lautenschlager *et al.*, *Neurology* **48**, 641 (1996)].
21. P. Giannakopoulos *et al.*, *J. Neuropathol. Exp. Neurol.* **55**, 1210 (1996).
22. J. Q. Trojanowski *et al.*, *Brain Pathol.* **3**, 45 (1993).
23. J. C. Vickers *et al.*, *Neuroscience* **62**, 1 (1994); J. C. Vickers *et al.*, *Exp. Neurol.* **141**, 1 (1996); J. C. Vickers, *Neuroscience* **78**, 629 (1997).
24. C. Miller *et al.*, *EMBO J.* **5**, 269 (1986); H. Ksiazek-Redding, D. W. Dickson, P. Davies, S. H. Yen, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3410 (1987); V. M. Y. Lee, L. Otvos Jr., J. Q. Trojanowski, *ibid.* **85**, 7384 (1988); K. S. Kosik *et al.*, *Neuron* **1**, 817 (1988); C. M. Wischik *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 4506 (1988); C. Banerjee *et al.*, *Brain Res.* **477**, 90 (1989); A. Delacourte *et al.*, *Acta Neuropathol.* **80**, 111 (1990); S. G. Greenberg and P. Davies, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 5827 (1990); M. L. Schmidt, V. M. Y. Lee, J. Q. Trojanowski, *Am. J. Pathol.* **136**, 1069 (1990); J. P. Brion, D. P. Hanger, A. M. Couck,
25. B. Anderton, *Biochem. J.* **279**, 831 (1991); V. M.-Y. Lee, B. J. Balin, L. Otvos Jr., J. Q. Trojanowski, *Science* **251**, 675 (1991); M. Goedert, M. G. Spillantini, N. J. Cairns, R. A. Crowther, *Neuron* **8**, 159 (1992).
26. F. Côté, J. F. Collard, J. P. Julien, *Cell* **73**, 35 (1993); Z. Xu, L. C. Cork, J. W. Griffin, D. W. Cleveland, *ibid.*, p. 23; M. E. Gurney *et al.*, *Science* **264**, 1772 (1994); M. K. Lee, J. R. Marszalek, D. W. Cleveland, *Neuron* **13**, 975 (1994); M. E. Ripps, G. W. Huntley, P. R. Hof, J. W. Gordon, J. H. Morrison, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 689 (1995); P. H. Tu *et al.*, *ibid.* **93**, 3155 (1996); M. Dal Canto and M. E. Gurney, *Acta Neuropathol.* **93**, 537 (1997).
27. B. M. Morrison, J. W. Gordon, M. E. Ripps, J. H. Morrison, *J. Comp. Neurol.* **373**, 619 (1996).
28. J. C. Vickers *et al.*, *J. Neurosci.* **14**, 5603 (1994).
29. T. Saitoh *et al.*, *Lab. Invest.* **64**, 596 (1991); H. Ischiropoulos *et al.*, *Arch. Biochem. Biophys.* **298**, 431 (1992); M. P. Mattson *et al.*, *J. Neurosci.* **12**, 376 (1992); M. F. Beal, B. T. Hyman, W. Koroshetz, *Trends Neurosci.* **16**, 125 (1993); J. S. Beckman, M. Carson, C. D. Smith, W. H. Koppenol, *Nature* **364**, 584 (1993); J. S. Beckman and J. P. Crow, *Biochem. Soc. Trans.* **21**, 330 (1993); M. A. Smith *et al.*, *Am. J. Pathol.* **145**, 42 (1994); M. F. Beal, *Ann. Neurol.* **38**, 357 (1995); G. Benzi and A. Moretti, *Neurobiol. Aging* **16**, 661 (1995); P. F. Good, P. Wemer, A. Hsu, C. W. Olanow, D. P. Perl, *Am. J. Pathol.* **149**, 21 (1996); J. P. Bolaños *et al.*, *J. Neurochem.* **68**, 2227 (1997); L. J. McIntosh, M. A. Trush, J. C. Troncoso, *Free Radical Biol. Med.* **23**, 183 (1997); M. A. Smith, P. L. Richey Harris, L. M. Sayre, J. S. Beckman, G. Perry, *J. Neurosci.* **17**, 2653 (1997).
30. H. Brody, *J. Comp. Neurol.* **102**, 511 (1955); A. D. Dayan, *Acta Neuropathol.* **6**, 85 (1970); E. J. Colon, *Psychiatr. Neurol. Neurochir.* **75**, 261 (1972).
31. P. D. Coleman and D. G. Flood, *Neurobiol. Aging* **8**, 521 (1987). The degree to which parameters of tissue fixation, neuronal shrinkage, and age-related changes in the volume of brain structures might be confounding factors when one assumes that decreased neuron density equates with decreased neuron number started to become apparent in the early 1980s, when a study by Haug *et al.* reported that, in fact, there was no neuron loss with aging in the human cerebral cortex [H. Haug, S. Kuhl, E. Mecke, N. L. Sass, K. Wasner, *J. Hirnforsch.* **25**, 353 (1984)].
32. A. H. Gazzaley, M. M. Thakker, P. R. Hof, J. H. Morrison, *Neurobiol. Aging*, in press. The aged monkeys analyzed in this study were not behaviorally characterized, and the analysis has yet to be extended to a large number of animals; thus, we are unable to determine the degree of individual variability in the NMDA receptor changes or the degree to which the receptor changes correlate directly with behavioral deficits.
33. P. R. Rapp and M. Gallagher, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 9926 (1996).
34. B. Pakkenberg and H. J. G. Gundersen, *J. Comp. Neurol.* **384**, 312 (1997).
35. Estimates of neuron loss in the hippocampus of elderly humans varies among studies. Although West and colleagues (16) demonstrated a loss in the hilus and subiculum, a recent analysis by Simic *et al.* [G. Simic, I. Kostovic, B. Winblad, N. Bodjanovic, *J. Comp. Neurol.* **379**, 482 (1997)] revealed correlations between age and neuron numbers in the CA1 and subiculum and severe decrease in neuron numbers in the dentate gyrus and subiculum only in AD cases. These discrepancies may be due to slight differences in the quantification method used and may also reflect the substantial degree of interindividual anatomical variability that exists in the human brain.
36. D. G. Flood and P. D. Coleman, *Neurobiol. Aging* **9**, 453 (1988); C. A. Barnes, in *Handbook of Neuropsychology*, F. Boller and J. Grafman, Eds. (Elsevier, Amsterdam, 1990), vol. 4, pp. 169-196; M. Gallagher and P. R. Rapp, *Annu. Rev. Psychol.* **48**, 339 (1997); P. R. Rapp, M. T. Kamsky, J. A. Roberts, *NeuroReport* **8**, 1923 (1997).
37. C. A. Barnes, M. S. Suster, J. Shen, B. J. McNaughton, *Nature* **388**, 272 (1997).



37. T. Crook *et al.*, *Dev. Neuropsychol.* **4**, 261 (1986).
38. M. S. Albert, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13547 (1996); — and M. B. Moss, in *Handbook of Biology of Aging*, E. L. Schneider, J. W. Rowe, T. E. Johnson, N. J. Holbrook, J. H. Morrison, Eds. (Academic Press, San Diego, CA, ed. 4, 1996), pp. 217–233; R. Fama *et al.*, *Arch. Neurol.* **54**, 719 (1997); A. Convit *et al.*, *Neurobiol. Aging* **18**, 131 (1997); C. R. Jack Jr. *et al.*, *Neurology* **49**, 786 (1997).
39. J. W. Rowe and R. L. Kahn, *Science* **237**, 143 (1987).
40. S. L. Vincent, A. Peters, J. Tigges, *Anat. Rec.* **223**, 329 (1989); J. Tigges, J. G. Herndon, A. Peters, *Neurobiol. Aging* **11**, 201 (1990); J. Bachevalier *et al.*, *ibid.* **12**, 99 (1991); A. Peters and C. Sethares, *Anat. Rec.* **236**, 721 (1993); A. Peters, D. Leahu, M. B. Moss, K. J. McNally, *Cerebr. Cortex* **4**, 621 (1994); Y. Geinisman, L. De Toledo-Morrell, F. Morrell, R. E. Heller, *Prog. Neurobiol.* **45**, 223 (1995); P. R. Rapp, in *Emotion, Memory and Behavior*, T. Nakajima and T. Ono, Eds. (Japan Scientific Societies Press, Tokyo, 1995), pp. 197–211; J. Tigges, J. G. Herndon, D. L. Rosene, *Acta Anat.* **153**, 39 (1995); A. Peters *et al.*, *J. Neuropathol. Exp. Neurol.* **55**, 861 (1996); J. Tigges, J. G. Herndon, D. L. Rosene, *Acta Anat.* **157**, 63 (1996); C. B. Y. Kim, L. P. Pier, P. D. Spear, *Anat. Rec.* **247**, 119 (1997).
41. W. T. Greenough, R. W. West, T. J. DeVoogd, *Science* **202**, 1096 (1978); D. G. Flood, S. J. Buell, G. Horwitz, P. D. Coleman, *Brain Res.* **402**, 205 (1987); F. L. F. Chang, K. R. Isaacs, W. T. Greenough, *Neurobiol. Aging* **12**, 517 (1991); L. M. Callahan and P. D. Coleman, *ibid.* **16**, 311 (1995).
42. A. Peters, *J. Comp. Neurol.* **371**, 153 (1996).
43. C. A. Barnes, *Trends Neurosci.* **17**, 13 (1994).
44. — and B. J. McNaughton, in *Psychobiology of Aging: Problems and Perspectives*, D. Stein, Ed. (Elsevier, Amsterdam, 1980), pp. 253–272; D. L. Deupree, D. A. Turner, C. L. Watter, *Brain Res.* **554**, 1 (1991); C. I. Moore, M. D. Browning, G. M. Rose, *Hippocampus* **3**, 57 (1993).
45. T. V. P. Bliss and T. Lomo, *J. Physiol. (London)* **232**, 331 (1973); R. G. M. Morris, E. Anderton, G. S. Lynch, M. Baudry, *Nature* **319**, 774 (1986); M. Meunier, J. Bachevalier, M. Mishkin, E. A. Murray, *J. Neurosci.* **13**, 5418 (1993); T. V. P. Bliss and G. L. Collingridge, *Nature* **361**, 31 (1993).
46. A. H. Gazzaley, S. J. Siegel, J. H. Kordower, E. J. Mufson, J. H. Morrison, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 3121 (1996).
47. M. E. Bach, R. D. Hawkins, M. Osman, E. R. Kandel, M. Mayford, *Cell* **81**, 905 (1995); M. Mayford, J. Wang, E. R. Kandel, T. J. O'Dell, *ibid.*, p. 891; K. Sakimura *et al.*, *Nature* **373**, 151 (1995); T. Abel *et al.*, *Cell* **88**, 615 (1997).
48. For example, see L. S. Higgins, D. M. Holtzman, J. Rabin, W. C. Mobley, B. Cordell, *Ann. Neurol.* **35**, 598 (1994); D. Games *et al.*, *Nature* **373**, 523 (1995); L. S. Higgins, J. M. Rodems, R. Catalano, D. Quon, B. Cordell, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 4402 (1995); E. Masliah *et al.*, *J. Neurosci.* **16**, 5795 (1996); M. C. Irizarry *et al.*, *ibid.* **17**, 7053 (1997); M. Jucker and D. K. Ingram, *Behav. Brain Res.* **85**, 1 (1997); J. Nalbantoglu *et al.*, *Nature* **387**, 500 (1997).
49. C. S. Woolley and B. S. McEwen, *J. Neurosci.* **12**, 2549 (1992); *J. Comp. Neurol.* **336**, 293 (1993); *J. Neurosci.* **14**, 7680 (1994).
50. A. H. Gazzaley, N. G. Weiland, B. S. McEwen, J. H. Morrison, *J. Neurosci.* **16**, 6830 (1996).
51. C. S. Woolley, N. G. Weiland, B. S. McEwen, P. A. Schwartzkroin, *ibid.* **17**, 1848 (1997).
52. H. Fillit *et al.*, *Psychoneuroendocrinology* **11**, 337 (1986); C. Berr, S. Lafont, B. Debuire, J. F. Dartigues, E. E. Beaulieu, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 13140 (1996); M. Tang *et al.*, *Lancet* **348**, 429 (1996); "The Role of Ovarian Hormones in Cognition and Dementia," S. J. Birge, Ed., *Neurology* **48** (suppl. 7), S1 (1997); C. Kawas *et al.*, *ibid.*, p. 1517; J. A. Roberts, K. V. K. Gilardi, B. Lasley, P. R. Rapp, *NeuroReport* **8**, 2047 (1997).
53. Y. Goodman, A. J. Bruce, B. Cheng, M. P. Mattson, *J. Neurochem.* **66**, 1836 (1996).
54. D. C. Sterio, *J. Microsc.* **134**, 127 (1984).
55. R. W. Williams and P. Rakic, *J. Comp. Neurol.* **278**,

- 344 (1988); *ibid.* **281**, 335 (1989); M. J. West, L. Slomianka, H. J. G. Gundersen, *Anat. Rec.* **231**, 482 (1991); M. J. West, *Neurobiol. Aging* **14**, 275 (1993).
56. We thank C. A. Barnes, C. Bouras, A. H. Gazzaley, P. Giannakopoulos, C. V. Mobbs, E. A. Nimchinsky, P. R. Rapp, and J. C. Vickers for their crucial contri-

butions and advice, and W. G. M. Janssen, A. P. Leonard, and R. S. Woolley for expert technical assistance. Research in our laboratory was supported by NIH grants AG05138 and AG06647, the Human Brain Project MHDA52145, the Charles A. Dana Foundation, and the Brookdale Foundation.

The Endocrinology of Aging

Steven W. J. Lamberts,* Anniewie W. van den Beld,
Aart-Jan van der Lely

Most aging individuals die from atherosclerosis, cancer, or dementia; but in the oldest old, loss of muscle strength resulting in frailty is the limiting factor for an individual's chances of living an independent life until death. Three hormonal systems show decreasing circulating hormone concentrations during normal aging: (i) estrogen (in menopause) and testosterone (in andropause), (ii) dehydroepiandrosterone and its sulphate (in adrenopause), and (iii) the growth hormone/insulin-like growth factor I axis (in somatopause). Physical changes during aging have been considered physiologic, but there is evidence that some of these changes are related to this decline in hormonal activity. Hormone replacement strategies have been developed, but many of their aspects remain controversial, and increasing blood hormone levels in aging individuals to those found during mid-adult life has not been uniformly proven to be safe and of benefit.

The average length of human life is currently 75 to 78 years and may increase to 85 years during the coming two decades (1), but is not clear whether these additional years will be satisfying to live. Most data indicate a modest gain in the number of healthy years lived but a far greater increase in years of compromised physical, mental, and social function (2). The number of days of restricted activity and the number of admissions to hospitals and nursing homes sharply increases after age 70 (3). One U.S. health interview survey indicates that, at present, more than 25 million aging people suffer from physical impairment, whereas the number of people requiring assistance with activities of daily living increases from 14% at age 65 to 75 to 45% in those over 85 years old (4).

Aging and Physical Frailty

Throughout adult life, all physiological functions gradually decline (5). There is a diminished capacity for cellular protein synthesis, a decline in immune function, an increase in fat mass, a loss of muscle mass and strength, and a decrease in bone mineral density (5). Most elderly individuals will die from atherosclerosis, cancer, or dementia; but in an increasing number of the

healthy oldest old, loss of muscle strength is the limiting factor that determines their chances of living an independent life until death.

Age-related disability is characterized by generalized weakness, impaired mobility and balance, and poor endurance. In the oldest old, this state is called physical frailty, which is defined as "a state of reduced physiological reserves associated with increased susceptibility to disability" (6). Clinical correlates of physical frailty include falls, fractures, impairment in activities of daily living, and loss of independence; falls contribute to 40% of admissions to nursing homes (7).

Loss of muscle strength is an important factor in the process of frailty. Muscle weakness can be caused by aging of muscle fibers and their innervation, osteoarthritis, and chronic debilitating diseases (8). However, a sedentary lifestyle and decreased physical activity and disuse are also important determinants of the decline in muscle strength. In a study of 100 frail nursing home residents (average age 87 years), lower-extremity muscle mass and strength were closely related (9). Supervised resistance exercise training (for 45 min three times per week for 10 weeks) doubled muscle strength and significantly increased gait velocity and stair-climbing power. This demonstrates that frailty in the elderly is not an irreversible effect of aging and disease but can be reduced and perhaps even prevented (9). Also, among nondisabled elderly people living in the

The authors are in the Department of Medicine, Erasmus University, Rotterdam, Netherlands.

*To whom correspondence should be addressed at the Department of Medicine, University Hospital Dijkzigt, 40 Dr. Molewaterplein, 3015 GD Rotterdam, Netherlands. E-mail: lamberts@inw3.azr.nl